

# VARIABILITY OF VEGETATIVE FLUSH COLORS IN *ZAMIA* (CYCADALES)

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## ABSTRACT

The color of new vegetative leaf flushes can vary among species of cycads. It is often used as a diagnostic character in taxonomy and is one of the most appreciated features of the group in ornamental horticulture. However, little is understood about the variability of flush color within species and wild populations. This paper discusses variability in flush color within the genus *Zamia* and uses a comprehensive ex situ conservation collection of three *Zamia* species (*Z. angustifolia*, *Z. integrifolia*, and *Z. lucayana*) collected from six islands in The Bahamas (Abaco, Andros, New Providence, Eleuthera, Grand Bahama, and Long Island) as a case study to examine this variability between species, between and within populations, and within progeny of individual mother plants. Flush color was found to be exclusively brown in *Z. angustifolia* and green in *Z. lucayana*. For *Zamia integrifolia*, flush color was exclusively green in seedlings derived from Abaco, Grand Bahama, and Eleuthera, but was variable in seedlings derived from Andros in New Providence, where both colors were present. On these two islands, flush color varied between and within populations, as well as within progeny of individual mother plants.

The observed variability in flush colors suggests that a large number of *in situ* observations from multiple plants in several populations would be required to determine if a particular flush color character state is fixed and therefore useful as a diagnostic character. This can only be done through long term study of *in situ* populations, as leaf production is typically seasonal and is not necessarily synchronized or universal within wild populations. Therefore, we recommend against the use of flush color as a diagnostic character in cycad taxonomy.

## RESUMEN

El color de las hojas nuevas de cicadas puede variar entre diferentes especies. A menudo se utiliza como carácter diagnóstico en taxonomía y es una de las características más apreciadas del grupo en horticultura ornamental. Sin embargo, se sabe muy poco sobre la variabilidad en el color de hojas nuevas dentro de las especies y poblaciones silvestres. En este artículo se discute la variabilidad en el color de hojas nuevas dentro del género *Zamia* y se usa una colección extensa de conservación ex situ de tres especies de *Zamia* (*Z. angustifolia*, *Z. integrifolia*, y *Z. lucayana*) colectadas de seis islas de las Bahamas (Abaco, Andros, New Providence, Eleuthera, Grand Bahama, y Long Island) como un estudio de caso para examinar esta variabilidad entre especies, en y entre poblaciones, y en descendientes de la misma planta madre. El color de hojas nuevas fue exclusivamente café en *Z. angustifolia* y verde en *Z. lucayana*. Para *Zamia integrifolia*, el color de hojas nuevas fue exclusivamente verde en plántulas derivadas de Abaco, Grand Bahama, y Eleuthera, pero variables en plántulas de Andros y New Providence, donde ambos colores estaban presentes. En estas dos islas, el color de hojas nuevas variaba en y entre poblaciones así como también entre descendientes de una misma planta madre.

La variabilidad observada sugiere que un número grande de observaciones *in situ* de múltiples plantas en múltiples poblaciones serían requeridas para determinar si el estado de carácter para el color de hojas nuevas es un carácter fijo que pueda servir como carácter diagnóstico. Esto solo se podría hacer a través de estudios de poblaciones *in situ* a largo plazo porque la producción de hoja nueva ocurre por temporadas y no necesariamente está sincronizado o es universal en las poblaciones silvestres. Por estas razones recomendamos que el color de las hojas nuevas no se utilice como carácter diagnóstico en la taxonomía de las cicadas.

## INTRODUCTION

Cycads are highly appreciated as ornamental plants due to their ease of culture, long life span, and distinctive appearance. They reproduce by means of pollen and seed cones that are always produced on separate plants (i.e. they are dioecious), and can be quite distinctive in some species. The seed cones in particular are one of the

most appreciated ornamental characteristics of cycads. They can be quite large in some species, as exemplified by *Lepidozamia peroffskyana* Regel (Fig. 1A), that has cones reaching 1 m in length and 40 kg in weight (Norstog & Nicholls 1997:235) and are considered the largest cones in the plant kingdom. In addition to attaining impressive dimensions in some species, seed cones can often be quite colorful, particularly in the African genus *Encephalartos* as exemplified by the bright orange cones of *E. ferox* G. Bertol (Fig. 1B).

In addition to being appreciated for their highly ornamental strobili, cycads are also highly prized for their foliage. Cycads have distinctive pinnately compound leaves which are held in a rigid crown. Although cycad leaves are relatively conservative in their morphology compared to some other plant groups (Norstog & Nicholls 1997:49), they can be quite variable in terms of texture, leaflet arrangement, and the color of new leaf flushes. This variation in foliage is best exemplified by the neotropical genus *Zamia*, which is considered to be the most morphologically diverse genus in the order Cycadales.

*Zamia*, consisting of 75 accepted species (<http://cycadlist.org>), is the most species-rich cycad genus in the New World and has the broadest geographic distribution, ranging from Southern Georgia to Bolivia. Throughout its vast geographic range, the genus occupies a variety of diverse environments ranging from open, desert-like environments, to dense rainforests in some of the rainiest places on earth. Adaptation to these different environments has resulted in remarkable variation in leaf form within the genus.

Most species of *Zamia* may hold multiple leaves per crown, but some species, such as *Z. meermanii* Calonje, typically hold only 1–3 leaves per crown. Leaflets in *Zamia* vary in texture from soft and paper-like as in *Z. vazquezii* D.W. Stev., Sabato & De Luca (Fig. 2A), to rigid and cardboard-like, as in *Z. encephalartoides* D.W. Stev. (Fig. 2B), a desert-dwelling species from Colombia. Some species, such as *Z. hamannii* A.S. Taylor, J.L. Haynes & Holzman (Fig. 2C), have broad, deeply-veined leaflets.

Leaflets vary in size from the diminutive leaflets of *Z. pygmaea* Sims, which may represent the smallest leaflets in the cycadales (Fig. 2D), to the large paddle-like leaflets of *Z. imperialis* A.S. Taylor, J.L. Haynes & Holzman which at up to 75 cm in length and 21 cm in width (Taylor et al. 2008) may be the largest leaflets found in living cycads (Fig. 2E). Leaflets also vary in arrangement from being widely spaced along the rachis, such as those of *Z. imperialis*, to closely spaced and overlapping, such as in *Z. chigua* Seem. (Fig. 2F).

In addition to the remarkable diversity in leaflet shape, size, texture and arrangement, the genus *Zamia* exhibits the greatest diversity in the color of new vegetative flushes. The color of these young developing leaves is perhaps one of the most ornamentally appreciated features of the genus. New leaves are produced in an array of different colors, including bright orange (*Z. pyrophylla* Calonje, D.W. Stev., & A.Lindstr.; Fig. 3A), light green (*Z. nesophila* A.S. Taylor, J.L. Haynes & Holzman; Fig. 3B), reddish brown (*Z. lacandona* Schutzman & Vovides; Fig. 3C), white (*Z. stevensonii* A.S. Taylor & Holzman; Fig. 3D), reddish-orange (*Z. hamannii*; Fig. 3E), and reddish-brown (*Z. purpurea* Vovides, J.D. Rees & Vázq. Torres; Fig. 3F).

Flush color appears to be a fixed trait in many *Zamia* species and is sometimes used as a diagnostic taxonomic character. For example, *Z. hamannii* is distinguished from closely related species by its reddish-orange to rosy-pink flushes (Fig 3E; Taylor et al. 2008), whereas *Z. stevensonii* (Fig. 3D) is distinguished from its putative sister species *Z. elegantissima* Schutzman, Vovides & R.S. Adams by its white flushes compared to the bright yellow flushes of *Z. elegantissima* (Taylor & Holzman 2012).

Although flush color appears to be fixed in many species, it can also be variable in some species. For example, a wide array of colors has been observed in cultivated individuals of some species such as *Z. standleyi* Schutzman and *Z. vazquezii* (Broome 2002). As the exact provenance and pedigree of ornamentally cultivated cycads is often unknown, it is unclear whether the variation observed in some cultivated species is the result of source germplasm derived from separate populations with distinct flush colors, or if this variation can be found within individual wild populations.

Little has been published regarding the variability of flush color in wild populations of *Zamia* other than Schutzman's (1984) report of separate populations of *Z. splendens* Schutzman (currently considered synonym of *Z. katzeriana* (Regel) E. Rettig, sensu Nicolalde-Morejón et al. 2009) which produce either brilliant red or light green leaf flushes. However, the extent to which flush color may vary among and within populations of *Zamia* or even among the progeny of a single mother plant has not been previously examined.



FIG. 1. A. Mature cone of *Lepidozamia peroffskeyana* held by Dr. Andrew Vovides. B. Seed cone of *Encephalartos ferox*.

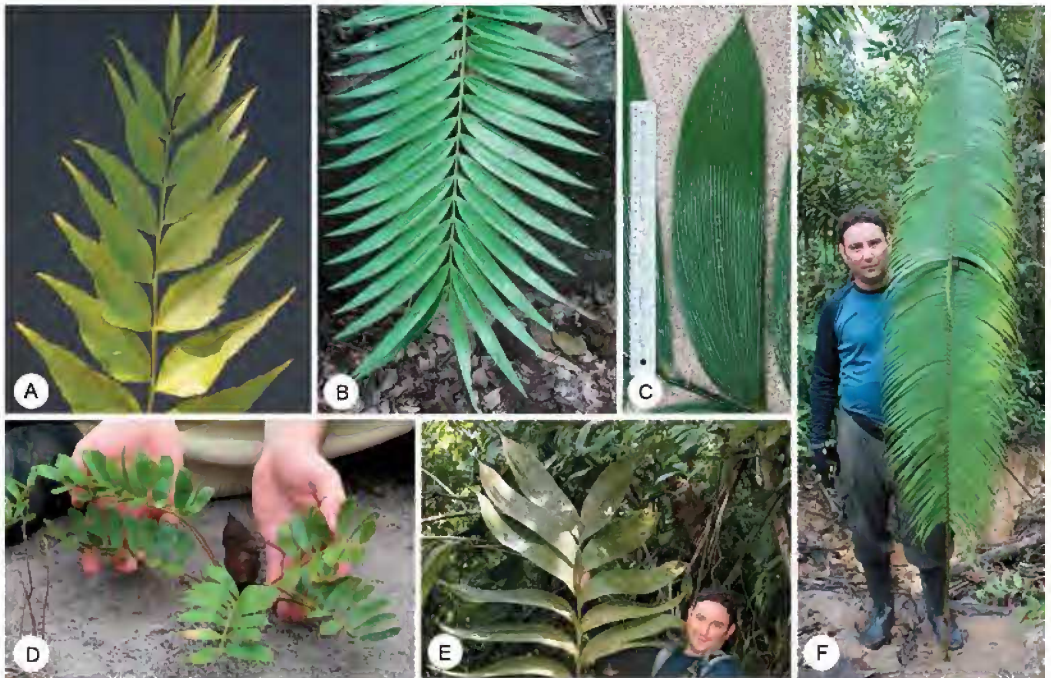


FIG. 2. Leaf variability in *Zamia*. Leaves of: A) *Z. vazquezii*, B) *Z. encephalartoides*, C) *Z. hamannii*, D) *Z. pygmaea*, E) *Z. imperialis*, and F) *Z. chigua*.





FIG. 3. Variability of flush color in *Zamia*. A) *Z. pyrophylla*, B) *Z. nesophila*, C) *Z. lacandona*, D) *Z. stevensonii*, E) *Z. imperialis*, F) *Z. purpurea*, G) *Z. amplifolia*, H) *Z. integrifolia* from New Providence, Bahamas displaying variable flush color in two plants derived from same mother plant.

Since flush color is often used as a diagnostic character, understanding flush color variability could have taxonomic implications. Similarly, understanding the variability in flush colors may inform future selective breeding of desirable flush colors for ornamental horticulture.

In this paper we explore variability of flush color by examining a taxonomically and geographically comprehensive ex-situ conservation collection of Bahamian *Zamia* plants cultivated at Montgomery Botanical Center (Coral Gables, Florida, USA). This collection was developed from 2009–2011 as part of a conservation genetics project focused on *Zamia lucayana* (see Calonje et al. 2013).

#### MATERIALS AND METHODS

Seeds were collected from wild populations to establish ex-situ conservation collections at Montgomery Botanical Center (MBC) and The Retreat of Bahamas National Trust (Nassau, New Providence, The Bahamas). Sampling encompassed the entire known distribution range for the genus in The Bahamas, including multiple populations on six different islands (Abaco, Andros, Eleuthera, Grand Bahama, New Providence, and Long Island) and representing three different species according to current species delimitations (Osborne et al. 2012). The three sampled species were: *Zamia angustifolia* from Eleuthera, *Z. lucayana* from Long Island, and *Zamia integrifolia*, which as currently circumscribed has a wide distribution including Abaco, Andros, Eleuthera, Grand Bahama, and New Providence (Fig. 4). *Zamia angustifolia* is distinguished by having very narrow leaflets, whereas *Z. lucayana* has the broadest leaflets. *Zamia integrifolia* has leaflets that vary in width on different islands; populations from Andros, New Providence and Eleuthera have narrower leaflets and populations from Grand Bahama and Abaco have broader leaflets (Fig. 5).

Sampling of seeds followed protocols developed at Montgomery Botanical Center that aim to maximize the genetic diversity of the ex-situ collections by sampling seeds from up to five mother plants per population (see Walters 2003). Seeds derived from individual mother plants, representing half siblings, are kept separate and provided their own accession number.

Seeds were germinated and seedlings cultivated at MBC. Flush color was documented on plants with new leaves during the period of 2012–2013. New vegetative flushes were observed on a total of 1133 seedlings comprising 62 separate accessions derived from a total of 14 wild populations.

The distribution of flush color in the seedlings was analyzed using JMP 10.0.2 statistical software (SAS Institute, Inc, Cary, North Carolina, USA). Contingency table analysis was used to detect variation in leaf color distribution across islands and across species. Following significant contingency table homogeneity tests, binomial analyses were used for detailed comparisons among species, populations, accessions, and islands.

#### RESULTS

##### Flush color by species and island

At the species level, flush color was exclusively brown in *Z. angustifolia* (from 2 populations in Eleuthera), exclusively green in *Z. lucayana* (from 3 populations in Long Island), and variable in *Z. integrifolia*, where exclusively green flushes occur on Abaco, Grand Bahama, and Eleuthera, and both colors occur on Andros and New Providence (Fig. 3). It should be noted that species delimitation in Caribbean *Zamia* is particularly unclear, and species determinations are tentative pending ongoing genetic studies of Caribbean *Zamia* (e.g., Calonje et al. 2013; Salas-Leiva et al. 2013; Meerow et al. 2012) that seek to clarify the genetic relationships among these populations and shed some light on their taxonomy and nomenclature.

Green leaf flushes were observed on seedlings from all six islands, whereas brown flushes were limited to seedlings from New Providence, Andros, and northern Eleuthera (Figs. 6–8). The three islands where brown flushes occur are adjacent to each other and were part of a single landmass known as Paleoprovidence in the Pleistocene when sea levels were lower. Along these three islands, there appears to be a longitudinal gradient in flush color proportion, as seedlings of *Z. angustifolia* from northern Eleuthera were entirely brown emergent (100% brown), those from New Providence mostly brown (85% brown), and those from Andros mostly green (33% brown). Contingency table analysis showed significant variation among islands ( $p < 0.0001$ ) and species ( $p < 0.0001$ ) (Tables 1, 2).

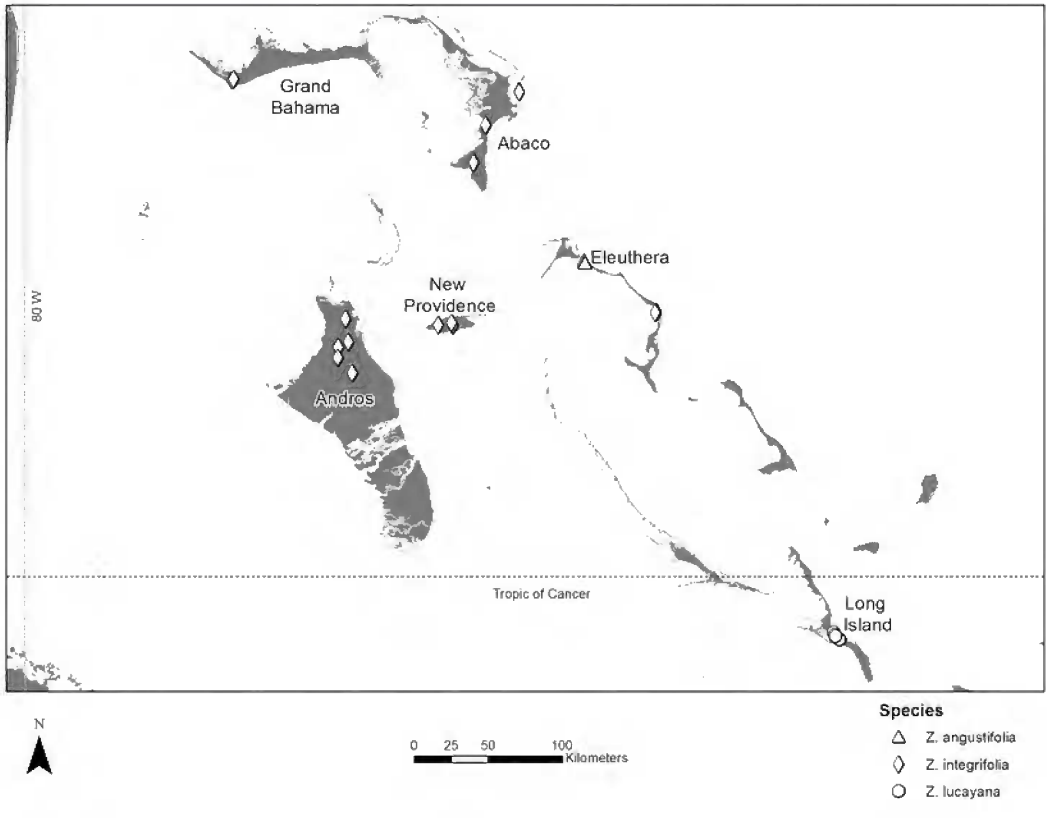


FIG. 4. *Zamia* collection sites in The Bahamas.

### Flush Color by Population

Of the three Bahamian species, only *Z. integrifolia* exhibited variation in flush color, and only on the islands of Andros and New Providence (Figs. 6–7), where variation was found among populations, within populations, and within progeny of individual mother plants which were treated as separate accessions (Fig. 7). On Andros, observed *Zamia* populations were restricted to North Andros, and only the southernmost sampled populations yielded offspring with brown-emergent leaves (Fig. 7). In New Providence, only a small number of accessions yielded offspring with green flushes. Contingency table analysis showed significant variation among populations ( $p < 0.0001$ ) (Table 3 for detailed comparisons).

### DISCUSSION

Flush color in Bahamian *Zamia* species appears to be genetically determined, as variation in flush color was found within wild populations on Andros and New Providence, and within the progeny of individual mother plants cultivated under identical conditions in MBC's ex-situ conservation collection. Plants with different flush colors from both islands were otherwise consistent in vegetative morphology.

Cycads are diploid, meaning that they have two sets of homologous chromosomes which may carry two different forms of a particular gene (alleles) at the same location. We have observed only green and brown leaf flushes on Bahamian *Zamia*, indicating that there may be at least two different alleles in a single gene for flush color present in Bahamian *Zamia* populations. The variability observed suggests that the alleles for particular flush colors may be fixed in some species, populations, and half-sibling cohorts, but polymorphic in others.

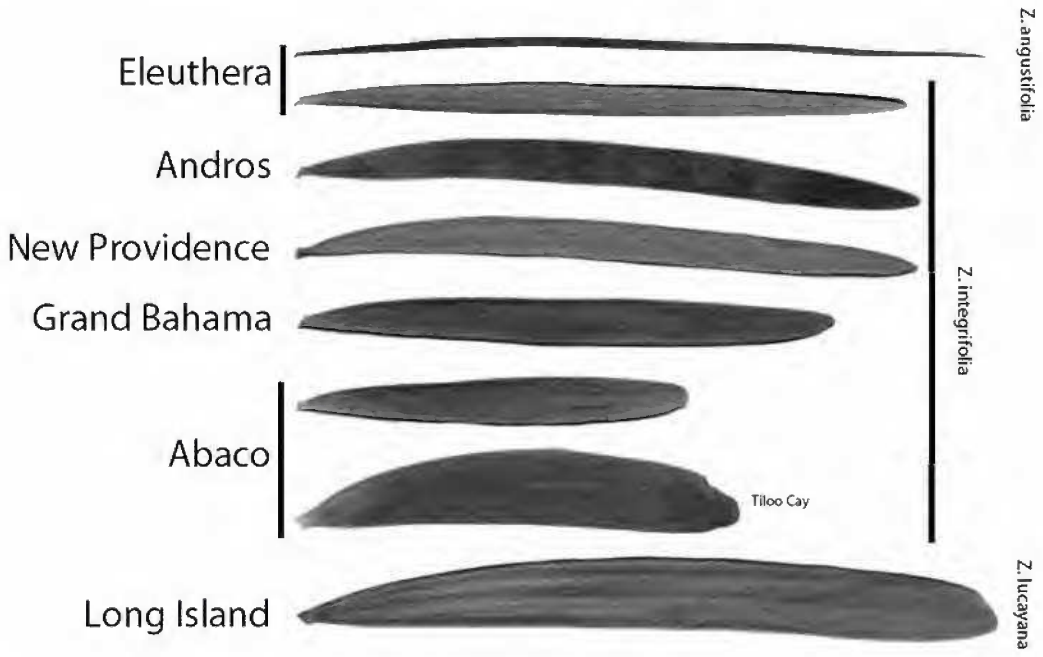


Fig. 5. Characteristic leaflet shapes of Bahamian *Zamia* populations.

TABLE 1. Numbers of brown and green emergent plants on different islands in the Bahamas.

Island	# Brown emergent	# Green emergent	Lower 95% CL* for % Brown emergent	Upper 95% CL* for % Brown emergent
Abaco	0	280	0.00%	1.31%
Long Island	0	192	0.00%	1.90%
Eleuthera	0	158	0.00%	2.31%
Grand Bahama	0	138	0.00%	2.64%
Andros Island	49	224	13.58%	23.03%
New Providence	124	23	77.45%	89.82%
Eleuthera	176	0	97.93%	100.00%

\* CL = Confidence Limit

TABLE 2. Numbers of brown and green emergent plants for different *Zamia* species in the Bahamas.

Species	# Brown emergent	# Green emergent	Lower 95% CL* for % Brown emergent	Upper 95% CL* for % Brown emergent
<i>Zamia lucayana</i>	0	192	0.00%	1.90%
<i>Zamia integrifolia</i>	173	823	15.07%	19.87%
<i>Zamia angustifolia</i>	176	0	97.93%	100.00%

\* CL = Confidence Limit

The differences in allele frequency among *Zamia* populations are likely the result of a combination of evolutionary forces including natural selection, genetic drift (particularly in small populations), and gene flow between populations (migration).

Assuming this trait is controlled by a single gene, it is not known whether a particular allele for flush color



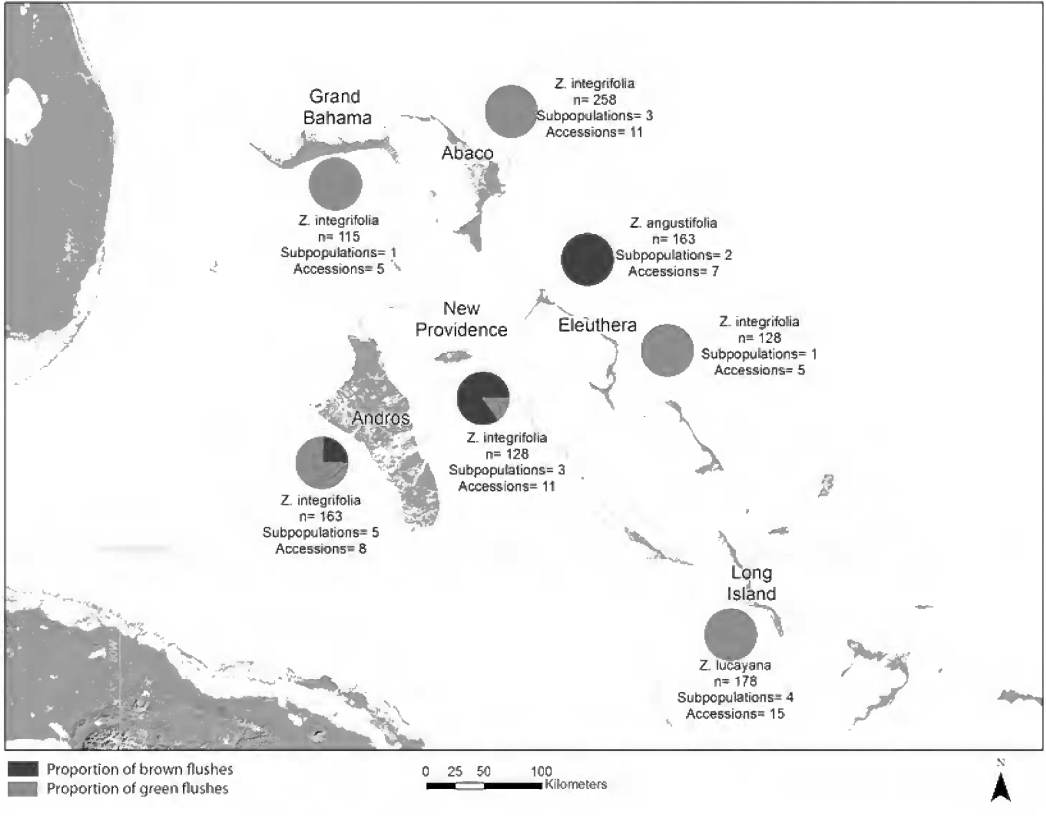


FIG. 6. Flush color distribution by species and island. Every island is represented by a single species except Eleuthera, which has separate populations of *Z. angustifolia* and *Z. integrifolia* with distinct flush colors.

may be dominant over the other, or whether this dominance may be consistent among different taxa. As the ex-situ collection at MBC is now beginning to reach reproductive maturity, the inheritance patterns of flush color may be determined by conducting controlled crosses between different plants and analyzing the flush color proportions of the resulting progeny. Caribbean species of *Zamia* are ideal for experimental crossing, because they can reach reproductive maturity in two to three years, which is relatively fast compared to other cycad species which may need a decade or longer to reach reproductive maturity.

It is not clear whether the brown flush color offers a selective advantage to cycads, but there are several possible hypotheses suggesting an advantage to brown or reddish flushes. The brown tint is believed to be produced by anthocyanins which may have fungicidal properties, protect leaves against UV damage, or make them inconspicuous to herbivores (Queenborough et al. 2013).

#### CONCLUSIONS

Flush color in Bahamian zamias varies geographically as well as at the species, population, and progeny levels, with color appearing to be fixed in some populations and variable in others. This variability suggests that to determine whether flush color is a fixed and therefore a diagnostic character for a given species, one would have to record flush color data from multiple individuals in each population and sample multiple populations. This is challenging data to collect because leaf production is seasonal and may not be synchronized among populations of a single species or individual plants within a population. Furthermore, a proportion of the population may not produce any leaves during a particular growing season, so collecting enough data to determine this would require long term study. Even if a fixed flush color were observed for a large sample of wild plants, at



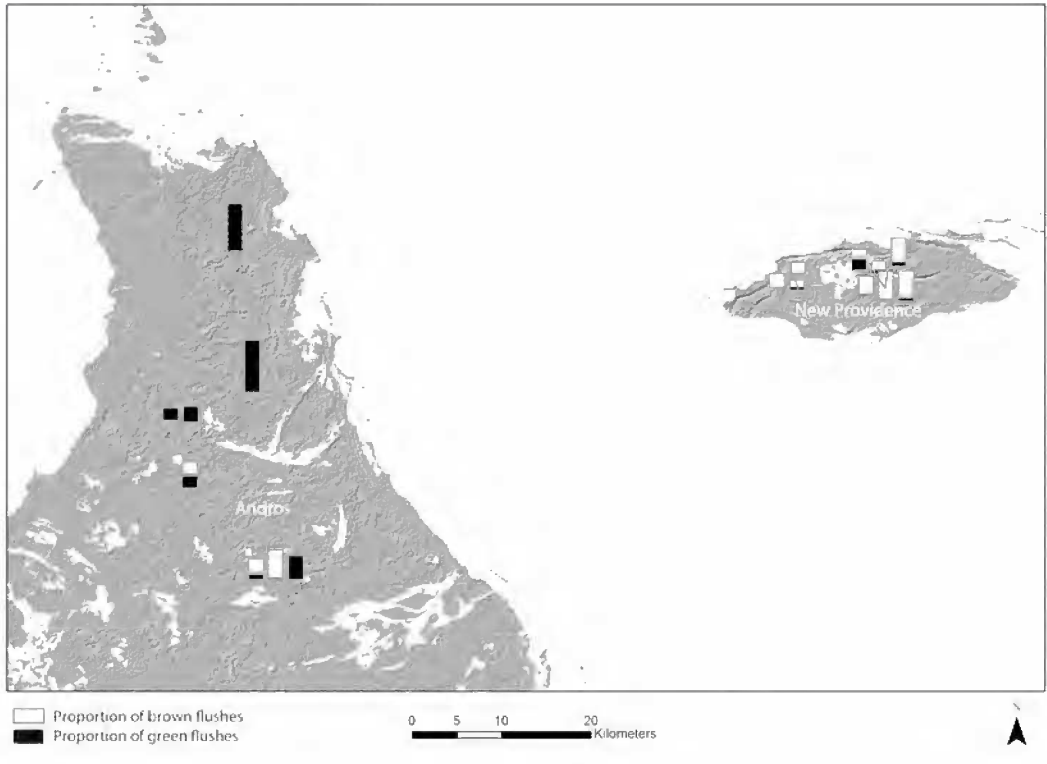


Fig. 7. Flush color of *Z. integrifolia* by accession in northern Andros and New Providence. Each bar represents a sampled accession, the height of the bar depending on the number of seedlings sampled from each accession, and the colors representing the proportion of flush colors found in the seedlings sampled from each accession.

TABLE 3. Numbers of brown and green emergent plants for different *Zamia* populations in the Bahamas.

Species	Population	# Brown emergent	# Green emergent	Lower 95% CL* for % Brown emergent	Upper 95% CL* for % Brown emergent
<i>Zamia lucayana</i>	13	0	192	0.00%	1.90%
<i>Zamia integrifolia</i> (Eleuthera)	6	0	158	0.00%	2.31%
<i>Zamia integrifolia</i> (Grand Bahama)	1	0	138	0.00%	2.64%
<i>Zamia integrifolia</i> (Abaco)	2	0	130	0.00%	2.80%
<i>Zamia integrifolia</i> (Abaco)	3	0	90	0.00%	4.02%
<i>Zamia integrifolia</i> (Abaco)	4	0	60	0.00%	5.96%
<i>Zamia integrifolia</i> (Andros Island)	10	0	58	0.00%	6.16%
<i>Zamia integrifolia</i> (Andros Island)	7	0	34	0.00%	10.28%
<i>Zamia integrifolia</i> (Andros Island)	11	9	9	26.02%	73.98%
<i>Zamia integrifolia</i> (Andros Island)	12	31	22	44.13%	71.86%
<i>Zamia integrifolia</i> (New Providence)	8	97	21	74.09%	88.63%
<i>Zamia integrifolia</i> (New Providence)	9	27	2	77.23%	99.15%
<i>Zamia angustifolia</i>	5	176	0	97.93%	100.00%

\* CL = Confidence Limit

present it would be difficult to use this character state diagnostically in the absence of similar data available for other species.

Although a large number of leaf flushes were observed in this study, the flush color proportions reported for a given wild population are based on the progeny of a relatively small number of sampled mother plants,

and are not necessarily representative of the populations as a whole. Consequently, we believe that flush color information should be included in cycad species descriptions but should not be used as a diagnostic character for taxonomy.

Flush color is variable in some species and appears to be genetically determined. Future studies to determine the heritability of this characteristic would be useful to inform selective breeding of cycads to emphasize desirable flush colors for ornamental horticulture.

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#### REFERENCES

- BROOME, T. 2002. A celebration of red and brown emergent cycads. *Cycad Newslett.* 25:4–7.
- CALONJE, M., A.W. MEEROW, L. KNOWLES, D. KNOWLES, M.P. GRIFFITH, K. NAKAMURA, & J. FRANCISCO-ORTEGA. 2013. Cycad biodiversity in the Bahamas Archipelago and conservation genetics of the threatened *Zamia lucayana* (Zamiaceae). *Oryx* 47:190–198.
- CYCADLIST.ORG. The world list of cycads. Accessed 03 Feb 2014. <http://cycadlist.org>
- MEEROW, A.W., J. FRANCISCO-ORTEGA, M. CALONJE, M.P. GRIFFITH, T. AYALA-SILVA, D.W. STEVENSON, & K. NAKAMURA. 2012. *Zamia* (Cycadales: Zamiaceae) on Puerto Rico: Asymmetric genetic differentiation and the hypothesis of multiple introductions. *Amer. J. Bot.* 99:1828–1839.
- NICOLALDE-MOREJÓN, F., A.P. VOVIDES, & D.W. STEVENSON. 2009. Taxonomic revision of *Zamia* in Mega-Mexico. *Brittonia* 61:301–335.
- NORSTOG, K.J. & T.J. NICHOLS. 1997. *The biology of the cycads*. Cornell University Press, Ithaca, New York, U.S.A.
- OSBORNE, R., M. CALONJE, K.D. HILL, L. STANBERG, & D.W. STEVENSON. 2012. The world list of cycads. *Mem. New York Bot. Gard.* 106:480–510.
- QUEENBOROUGH, S.A., M.R. METZ, R. VALENCIA, & S.J. WRIGHT. 2013. Demographic consequences of chromatic leaf defence in tropical tree communities: Do red young leaves increase growth and survival? *Ann. Bot.* 112:677–684.
- SALAS-LEIVA, D.E., A.W. MEEROW, M. CALONJE, M.P. GRIFFITH, J. FRANCISCO-ORTEGA, K. NAKAMURA, D.W. STEVENSON, C.E. LEWIS, & S. NAMOFF. 2013. Phylogeny of the cycads based on multiple single-copy nuclear genes: Congruence of concatenated parsimony, likelihood and species tree inference methods. *Ann. Bot.* 112:1263–1278.
- SCHUTZMAN, B. 1984. A new species of *Zamia* L. (Zamiaceae, Cycadales) from Chiapas, Mexico. *Phytologia* 55:299–303.
- TAYLOR, A.S., J.L. HAYNES, & G. HOLZMAN. 2008. Taxonomical, nomenclatural and biogeographical revelations in the *Zamia skinneri* complex of Central America (Cycadales: Zamiaceae). *Bot. J. Linn. Soc.* 158:399–429.
- TAYLOR, A.S. & G. HOLZMAN. 2012. A new *Zamia* species from the Panama Canal Area. *Bot. Rev.* 78:335–344.
- WALTERS, T. 2003. Off-site collections. In: J. Donaldson, ed. *Cycads: Status survey and conservation action plan*. IUCN, Gland, Switzerland and Cambridge, UK. Pp. 48–53.